

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
 - TEXT CUT OFF AT TOP, BOTTOM OR SIDES
 - FADED TEXT
 - ILLEGIBLE TEXT
 - SKEWED/SLANTED IMAGES
 - COLORED PHOTOS
 - BLACK OR VERY BLACK AND WHITE DARK PHOTOS
-
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

Infection of Cynomolgus Monkeys with a Chimeric HIV-1/SIV_{mac} Virus That Expresses the HIV-1 Envelope Glycoproteins

John Li, *Carol I. Lord, William Haseltine, *Norman L. Letvin, and Joseph Sodroski

Laboratory of Human Retrovirology, Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston; and *New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts

Summary: Replication competent chimeric viruses that express the gag and pol proteins of SIV_{mac} and the env proteins of HIV-1 were made. One such chimeric virus, SHIV-4, that expresses the *vif*, *vpx*, *vpr*, and *nef* regulatory genes of SIV and the *tat* and *rev* regulatory genes of HIV-1 replicated efficiently in cynomolgus monkeys. This model system can be used to evaluate the efficacy of anti-HIV-1 vaccines directed at the envelope glycoproteins, anti-HIV-1 envelope glycoprotein antiserum or monoclonal antibodies, and anti-HIV-1 drugs designed to inhibit the *tat*, *rev*, or *env* functions. **Key Words:** Chimeric virus—HIV/SIV—Cynomolgus monkey.

Human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2) are the etiologic agents of AIDS in humans (1-3). These viruses are related to simian immunodeficiency viruses (SIV) that infect feral populations of sooty mangabeys, African green monkeys, and mandrills (for review, see refs. 4 and 5).

Development of a vaccine to prevent infection of HIV-1 requires a suitable animal model. The two animal models most commonly used, infection of chimpanzees with HIV-1 and infection of macaque monkeys with SIV, have limitations. HIV-1 does not replicate to high titers in chimpanzees, and infected chimpanzees do not develop immunodeficiency (6-10). Trials in chimpanzees are limited to a few animals as the species is endangered, available chimpanzees are few, and care is expensive.

Rhesus and cynomolgus macaque monkeys infected with the macaque strain of SIV (SIV_{mac}) do produce high titers of virus and do develop an AIDS-like syndrome (11-13). Differences exist, however, in the immune responses to the HIV-1 and SIV_{mac} envelope glycoproteins, which represent the principal targets for protective immunity (14-16). The major neutralizing antibodies in HIV-1-infected people are directed against two regions of the gp120 envelope glycoprotein. Antibodies against the HIV-1 third gp120 variable (V3) region have been shown to be protective (15). In contrast, the corresponding region of the SIV_{mac} envelope glycoprotein does not exhibit sequence variation among isolates and is not a target for neutralizing antibodies in infected macaques (17,18). A second group of neutralizing antibodies in HIV-1-infected humans is directed against the conserved, discontinuous gp120 region that binds the CD4 viral receptor (19-29). These antibodies recognize HIV-1 gp120 regions distinct from those of the SIV_{mac} gp120 glycoprotein recognized by antibodies from

Address correspondence and reprint requests to Dr. J. Sodroski at Dana Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, U.S.A.

Manuscript received May 1, 1992; accepted May 7, 1992.

infected macaques that neutralize multiple SIV strains (30–33). The differences between the antibodies that broadly neutralize HIV-1 and SIV strains are highlighted by the observation that such antibodies do not cross-neutralize (34).

To overcome these difficulties an attempt was made to create a chimeric virus between HIV-1 and SIV_{mac} that contains the HIV-1 envelope glycoproteins and is capable of replicating to high titers in macaque monkeys.

MATERIALS AND METHODS

Plasmid Constructions

The chimeric viruses were constructed using the infectious, pathogenic SIV_{mac}239 (*nef* open) virus (*gag*+, *pro*+, *pol*+, *vif*+, *vpx*+, *vpr*+, *tat*+, *rev*+, *env*+, *nef*+) (12,35) and the HXBc2 HIV-1 virus (*gag*+, *pro*+, *pol*+, *vif*+, *vpr*+, *tat*+, *rev*+, *vpu*–, *env*+, *nef*–) (36). All four chimeric viruses (designated SHIV) used in this study express the *gag*, *pro*, *pol*, *vif*, *vpr*, and *nef* proteins of SIV_{mac}239 (*nef* open) and the *tat*, *rev*, and *env* proteins of HIV-1 (HXBc2).

Each chimeric provirus clone was propagated in *E. coli* using two plasmids, one containing the 5' half of the provirus and one containing the 3' half of the provirus. The 5' proviral clones, derived from the p239 SpSp 5' plasmid (12), consisted entirely of sequences from the SIV_{mac}239 clone. The sequences from the 5' cellular flanking sequences to the unique *Sph* I site in the SIV_{mac}239 genome were cloned into a pBS(+) plasmid (Stratagene) modified to contain a unique *Cla* I site in the polylinker region. This 5' clone, which was used to generate the SHIV-1 and SHIV-2 chimeric viruses, contains the SIV_{mac}239 *tat* splice acceptor and *tat* initiation codon. Site-directed mutagenesis was used to create a modified 5' clone in which the SIV_{mac}239 *tat* splice acceptor and *tat* initiation codon were modified (Fig. 1). This modified 5' clone was used to generate the SHIV-3 and SHIV-4 chimeric viruses.

The 3' proviral clones consisted of *tat*, *rev* and *env* sequences derived from the HXBc2 HIV-1 isolate and the *nef* and 3' LTR sequences derived from the SIV_{mac}239 (*nef* open) isolate (35). In the SIV_{mac}239 (*nef* open) variant, the 93rd codon of *nef* is changed from a stop (TAA) to a Glu (GAA) codon, allowing production of a functional *nef* protein (35). The HIV/SIV_{mac} junction in the 3' proviral clones was formed by ligating the HIV-1 and SIV_{mac} segments using the *Rsr* II site, which was created by site-directed mutagenesis in both the HIV-1 and SIV_{mac}239 (*nef* open) sequences.

To allow efficient ligation of the 5' and 3' proviral halves, a unique *Sph* I site was introduced by site-directed mutagenesis into the HIV-1 region upstream of the HIV-1 *tat* gene. This *Sph* I site was positioned such that the HIV-1 *tat* splice acceptor sequences would be either included in or excluded from the 3' proviral clones. The 3' clone that included the HIV-1 *tat* splice acceptor was used to generate the SHIV-1 and SHIV-3 chimeric viruses, while the 3' clone lacking the HIV-1 *tat* splice acceptor was used to generate the SHIV-2 and SHIV-4 viruses (Fig. 1). Also, since the last few codons of the SIV_{mac} *vpr* gene are located 3' to the natural *Sph* I site in the 5' proviral clone, these codons were supplied by modification of the 3' proviral clone near the introduced *Sph* I site. Thus, the *vpr* reading frame would be restored upon ligation of the 5' and 3' proviral clones at the *Sph* I site (Fig. 1).

Transfection of CEMx174 Cells with Chimeric Proviruses

For transfection, 5 µg of the 5' and 3' proviral clones were digested with *Sph* I and other restriction enzymes that recognize the flanking sequences (*Cla* I for the 5' proviral clone and *Xho* I for the 3' proviral clone). The fragments containing the 5' and 3' proviral sequences were ligated. The ligation reaction was then mixed with 3×10^6 CEMx174 cells suspended in 1 ml of serum-free RPMI 1640 and 500 µg/ml DEAE-dextran. The cell-DNA suspension was incubated at 37°C for 1 h, after which the cells were washed with serum-free medium and resuspended in 10 ml RPMI 1640 with 10% fetal calf serum.

Reverse Transcriptase Assays

Virus production in transfected or infected cultures was monitored every 3–4 days by reverse transcriptase assays as described, using 1.5 ml of cell-free supernatant (37). After removing supernatants for reverse transcriptase assays, cells were resuspended in a sufficient amount of fresh medium to maintain the cell density between 10^5 and 10^6 cells/ml.

Infection of Cultured Monkey PBMCs

Typically, $2-4 \times 10^7$ peripheral blood mononuclear cells (PBMCs) were isolated from 15–30 ml whole blood from cynomolgus monkeys. Cells were isolated using Ficoll-Paque (Pharmacia) and resuspended in RPMI 1640 supplemented with 10% fetal calf serum and either phytohemagglutinin (PHA-C) (Boehringer-Mannheim) or concanavalin A (Con A, type IV, Sigma) at 5 µg/ml. Three to 5 days following PHA-C or Con A stimulation, the cells were washed and resuspended in RPMI 1640 with 10% fetal calf serum and 10 U/ml interleukin-2 (human recombinant, Boehringer-Mannheim). Two days later, PBMCs were infected with 1×10^5 reverse transcriptase units of virus derived from transfected CEMx174 cells. Three days after infection, PBMCs were washed and resuspended in fresh medium. Reverse transcriptase measurements in cell supernatants were made on days 4, 6, 9, and 13 following infection.

Preparation of Virus Stocks and TCID₅₀ Determination

Virus stocks for animal inoculation were prepared in cynomolgus monkey PBMCs and frozen as cell-free supernatants without additives at –70°C. The virus titer was determined by incubating 100 µl of thawed stocks, either undiluted or as 10-fold serial dilutions, in quadruplicate with 1×10^5 CEMx174 cells in 1 ml of medium. When cultures became confluent, cells were diluted 1/10. The wells were scored for the presence of syncytia after 2 weeks, and the 50% tissue culture infectious dose (TCID₅₀) in the virus stock calculated as described (38).

Immunoprecipitation of Infected Cultures

Approximately 2×10^6 CEMx174 cells were infected with HIV-1 (HXBc2 strain), SIV_{mac}239 (*nef* open), or chimeric viruses. The cultures were labeled overnight with [³⁵S]cysteine 1–2 days prior to the peak of syncytium formation, and cell lysates were precipitated either with serum from a HIV-1-infected AIDS patient or from a SIV_{mac}-infected rhesus macaque as described (30).

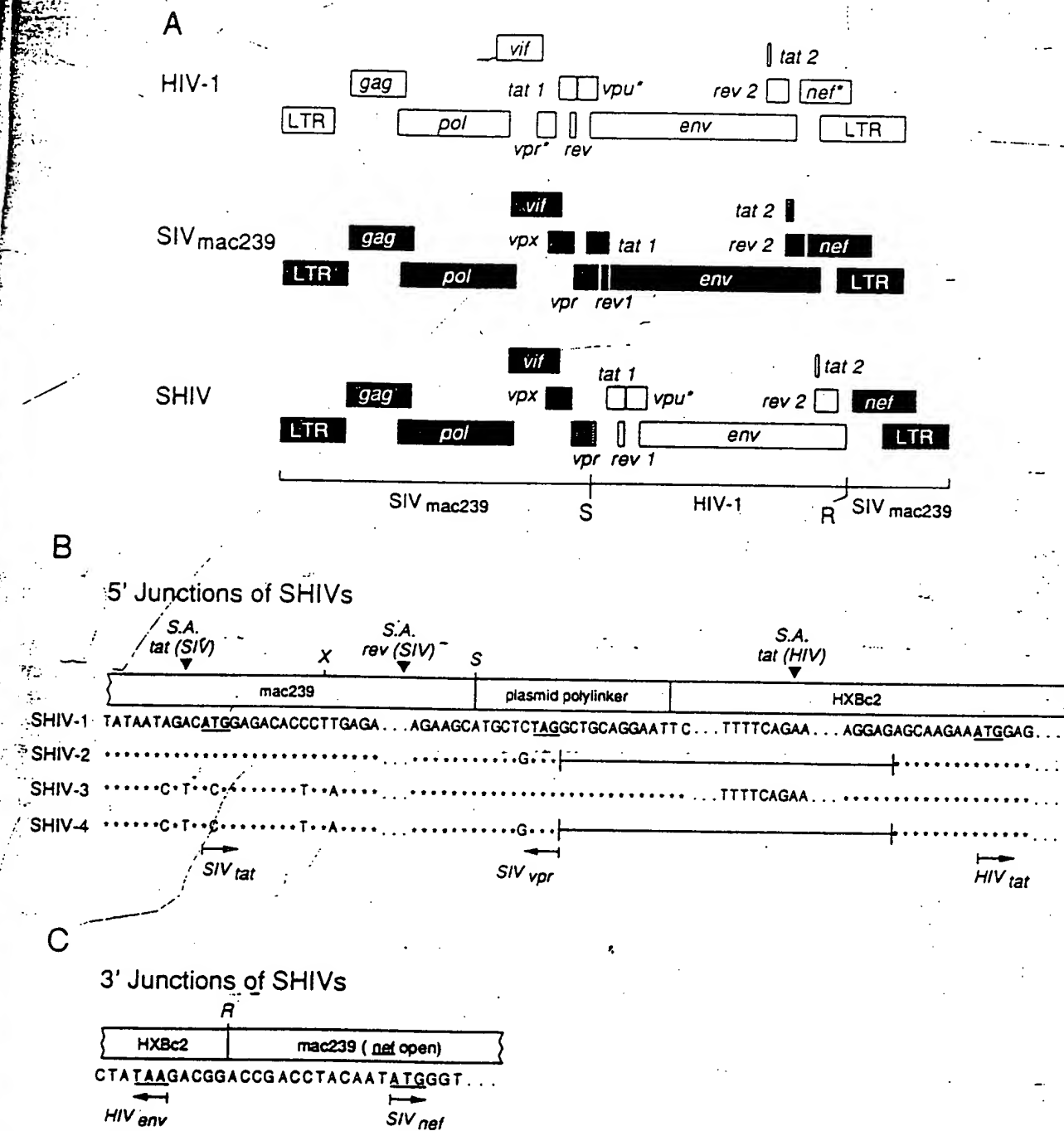


FIG. 1. Structure of the chimeric viruses. A: The genetic organization of the HIV-1 (HXBc2), SIV_{mac}239 (*nef* open), or SHIV chimeric viruses is shown, with HIV-1- or SIV_{mac}-specific sequences designated as white or black boxes, respectively. Genes that are defective in the strains utilized are denoted with an asterisk. The 5' SIV_{mac}/HIV-1 junction at the *Sph* I site (S) and the 3' HIV-1/SIV_{mac} junction at the *Rsr* II site (R) are shown. The stippled 3' end of *vpr* of the SHIV virus represents sequences derived from the HIV-1 portion of the chimera that reconstitute the SIV_{mac}239 *vpr*. B: The details of the 5' SIV_{mac}/HIV-1 junction near the *Sph* I site (S) are shown for each of the SHIV chimeric viruses. The positions of the splice acceptors (S.A.) for the SIV_{mac} *tat* and *rev* messages and for the HIV-1 *tat* message are shown above the figure, with the SIV_{mac} *tat* initiation codon, SIV_{mac} *vpr* stop codon and HIV-1 *tat* initiation codon underlined and labeled with arrows below. The asterisks denote sequence identity and the dots represent sequences not shown. The horizontal bars represent sequence deletions. The X marks the position of an *Xba* I site in the SHIV-3 and SHIV-4 sequences. C: The details of the 3' HIV-1/SIV_{mac}239 (*nef* open) junction near the *Rsr* II site (R) are shown. The stop codon for the HIV-1 *env* and the initiation codon for the SIV_{mac} *nef* are underlined and labeled with arrows.

Inoculation of Cynomolgus Monkeys with Chimeric Virus

Two male and two female cynomolgus monkeys (*M. fascicularis*) were inoculated intravenously with 1 ml of virus stock containing 7×10^3 TCID₅₀ of the SHIV-4 chimeric virus.

Virus Isolation from Inoculated Cynomolgus Monkeys

At 2 and 4 weeks following inoculation of cynomolgus monkeys, CD8-depleted, Con A-stimulated PBMCs were cultured from each animal and the level of SIV_{mac} gag p27 antigen in culture supernatants assessed as described (39). Culture supernatants positive for viral antigen were used to infect CEMx174 cells, which were labeled and used for immunoprecipitation as described above.

RESULTS

Chimeric Viruses

The goal of these studies was to create a replication-competent SIV/HIV-1 hybrid virus that expresses the HIV-1 envelope glycoproteins. The sequences used for the construction were derived from the pHXBc2 DNA, a clone prepared from the IIIB strain of HIV-1 (36), and the p239 SpSp 5' and p239 SpE3'/nef-open plasmids derived from the SIV_{mac}239 strain of virus (12,35). The SIV_{mac}239 viral DNA was selected to be one of the parents for the recombinant virus as injection of cynomolgus or rhesus monkeys with either purified viral DNA or virus derived from this DNA resulted in both high levels of viremia and an AIDS-like disease (12,35, 40).

Construction of the appropriate chimeric molecules was complicated by significant differences in the regulatory genes of the two viruses as well as the complex genetic organization of the primate immunodeficiency viruses (5,41,42). Both HIV-1 and SIV_{mac} encode the regulatory genes *vif*, *vpr*, *tat*, *rev*, and *nef*. The regulatory gene *vpu* is specified only by HIV-1 (43-48), whereas *vpx* is found only in HIV-2 or SIV (49-51). The strategy used for construction of chimeric viruses was to replace the *tat*, *rev*, and *env* sequences of SIV_{mac}239 by the corresponding sequences of HXB2. The resultant viruses should contain the LTR *gag*, *pol*, *vif*, *vpx*, *vpr* and *nef* of SIV_{mac} and *tat*, *rev*, and *env* of HIV-1.

The original chimeric virus, designated SHIV-1 (SIV-HIV-chimeric virus-1), contains two *tat* splice acceptor sequences. The 5' *tat* splice acceptor sequence is of SIV_{mac} origin whereas the 3' *tat* acceptor sequence is derived from HIV-1 sequences. To

minimize the possibility that the presence of two closely spaced splice acceptor sites might interfere with one another, derivatives of SHIV-1 were made that contain only the SIV_{mac} splice acceptor site (SHIV-2), only the HIV-1 splice acceptor site (SHIV-3) or neither splice acceptor site (SHIV-4) (Fig. 1). In the virus that lacks both *tat* splice acceptors, it is likely that the remaining SIV_{mac} *rev* acceptor substitutes for the *tat* acceptor.

Replication of Chimeric Viruses in Culture

The parental SIV_{mac}239 virus replicates well in the human CD4⁺ B/T cell hybrid line CEMx174 (52). To determine whether chimeric SHIV DNAs produce infectious virus, CEMx174 cells were transfected with the parental SIV_{mac}239 as well as SHIV recombinant DNAs. Virus replication was monitored by measurement of the amount of the viral DNA polymerase (reverse transcriptase) released into the culture medium.

The data of Fig. 2 show that virus is produced from cultures that are transfected with all five DNAs. However, significant differences in the rate of appearance of reverse transcriptase in the medium were noted using different DNAs. Significant virus replication was evident by 9 days post-transfection in cultures treated with either the parental SIV_{mac}239 DNA or the SHIV-2 or SHIV-4 DNAs (Fig. 2A). Detectable levels of reverse transcriptase were not present in the cultures transfected with SHIV-1 or SHIV-3 DNAs until day 13 post-transfection (Fig. 2B). The relative delay in appearance of virus in the supernatant of cultures transfected with SHIV-1 or SHIV-3 DNAs as compared to those transfected with SIV_{mac}239, SHIV-2 or SHIV-4 DNAs was observed in several independent experiments. Despite this reproducible delay, the rates of replication of all four chimeric viruses were indistinguishable when similar amounts of virus harvested from the supernatant fluids of the transfected cultures were used to reinfect CEMx174 cells (data not shown).

The ability of SHIV-2 and SHIV-4 viruses to initiate infection in primary PBMCs derived from cynomolgus monkeys was examined. For these experiments the SIV_{mac}239, SHIV-2, and SHIV-4 viruses harvested from the supernatant fluids of transfected CEMx174 cells were incubated with PHA-1- or Con A-activated monkey PBMCs. Three days after infection with these viruses, the PBMCs were washed and resuspended in fresh medium. Virus replication

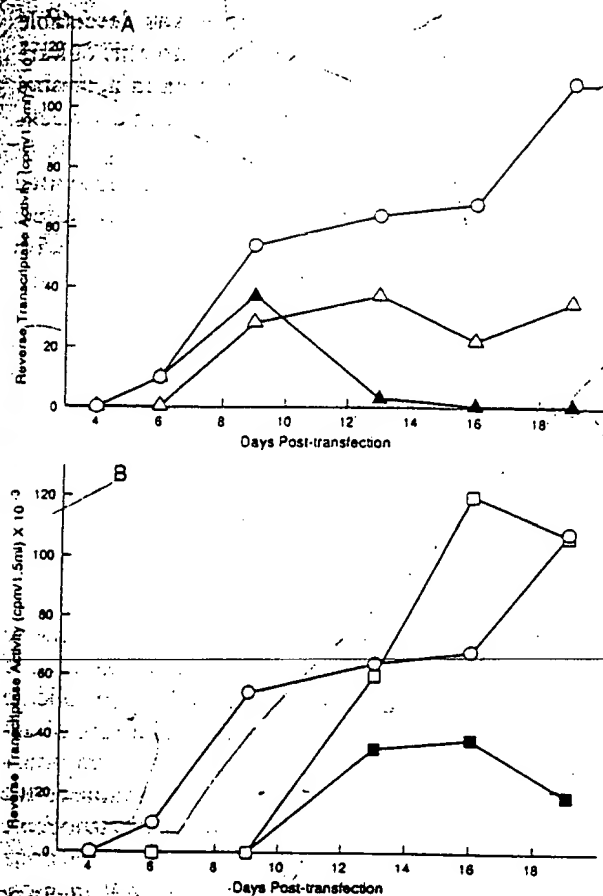


FIG. 2. Replication of chimeric viruses in CEMx174 lymphocytes. The reverse transcriptase activity in the supernatants of CEMx174 cells that had been transfected with proviral DNA of SIV_{mac}239 (*nef* open) (○) or chimeric viruses is shown. A: The CEMx174 cells were transfected with SHIV-2 (▲) or SHIV-4 (△) DNA. B: The CEMx174 cells were transfected with SHIV-1 (■) or SHIV-3 (□) DNA.

was measured by detection of reverse transcriptase activity in culture supernatant fluids.

The data of Table 1 show that all three viruses replicated well in cultures of PBMCs derived from cynomolgus monkeys. The rate of replication and amount of virus produced upon infection of the monkey PBMCs with either the SHIV-2 or SHIV-4 virus was similar to that obtained upon infection of the culture with SIV_{mac}239.

TABLE 1. Reverse transcriptase activity (cpm/1.5 ml × 10⁻³) in supernatants of cynomolgus monkey PBMCs

Virus	Days after infection			
	4	6	9	13
SIV _{mac} 239 (<i>nef</i> -open)	33	45	30	61
SHIV-2	96	82	41	28
SHIV-4	57	76	17	34

Chimeric Nature of the Recombinant Viruses

The SHIV chimeras should produce gag and pol products of SIV_{mac} and env proteins of HIV-1. The viral gag proteins of HIV-1 and SIV_{mac}239 can be distinguished by mobility differences on SDS-polyacrylamide gels, following precipitation with sera from HIV-1-infected humans or SIV_{mac}-infected monkeys. Such sera contain antibodies that cross-react with gag but not with env proteins (53). To determine whether the SHIV viruses that replicate in cynomolgus monkey PBMCs express both SIV_{mac} and HIV-1 proteins, viruses harvested from the supernatant fluids of infected PBMC cultures were used to infect CEMx174 cells. As controls, CEMx174 cells were infected with SIV_{mac}239 (*nef* open) and HIV-1 (HXBc2) viruses. The infected cells were labeled with [³⁵S]cysteine, lysed, and the viral proteins precipitated with serum from a HIV-1-infected AIDS patient or serum from a SIV_{mac}-infected macaque. The precipitates were analyzed on SDS-polyacrylamide gels.

The data of Fig. 3A show that, as expected, both the human and monkey sera recognize gag proteins of the parental HIV-1 and SIV viruses. These proteins can be distinguished from one another by electrophoretic mobility of both the capsid proteins (HIV-1 p24 and SIV_{mac} p27) and the gag precursor proteins (HIV-1 p55 and SIV_{mac} p58). The HIV-1 serum recognizes the gp160 and gp120 env glycoproteins present in CEMx174 cells infected with HIV-1 but not the env proteins of cells infected with SIV_{mac}239. The anti-SIV_{mac} serum recognizes the gp160 and gp130 env proteins present in cells infected with SIV_{mac}239 but not with the HIV-1 virus.

In these experiments the gag proteins present in cells infected with the SHIV-4 virus exhibited the electrophoretic mobility characteristic of SIV_{mac} capsid proteins. The env proteins of these extracts were recognized by the anti-HIV-1 but not the anti-SIV_{mac} serum. The electrophoretic mobilities of the env proteins present in cells infected with the SHIV-4 virus corresponded to those expected for the envelope glycoproteins of HIV-1. These experiments confirm that the SHIV-4 virus is chimeric and produces the gag proteins of SIV_{mac} and the env proteins of HIV-1.

Infection of Cynomolgus Monkeys

The ability of one of the chimeric viruses, SHIV-4, to replicate in cynomolgus monkeys was exam-

Shibata et al. (57) have also constructed a SIV_{mac}/HIV-1 chimeric virus that expresses the HIV-1 envelope glycoproteins. This virus is defective for both *vpr* and *nef*. The chimera described by Shibata et al. replicates in cultured monkey PBMCs. However, the rate of replication of this virus appears to be slower than that of the SIV_{mac} parental virus (57). The absence of *vpr* and *nef* and suboptimal splicing patterns may account, at least in part, for this delay.

The presence of the HIV-1 *tat* splice acceptor near the 5' SIV_{mac}/HIV-1 junction slowed the appearance of virus in cells transfected with the SHIV-1 or SHIV-3 DNA. It is likely that the presence of the HIV-1 *tat* splice acceptor results in inefficient expression of viral genes. The ability of virus harvested from CEMx174 cells transfected with proviruses containing the HIV-1 *tat* acceptor sequence to initiate efficient reinfection of CEMx174 cells probably indicates that selection for optimal splicing occurs rapidly in culture.

The experimental system described here (the infection of cynomolgus monkeys with the SHIV-4 virus) should provide a valuable model for study of the efficacy of anti-HIV-1 vaccines. The ability of such vaccines to induce protective immune responses in monkeys to infection by SHIV-4 should provide an indication of efficacy against viruses with HIV-1 envelope glycoproteins. The model can also be used to evaluate the ability of polyclonal and monoclonal antibodies to inhibit HIV-1 envelope function in animals. Therapeutics designed to inhibit the HIV-1 *tat*, *rev*, or *env* functions can also be evaluated in this model system.

Acknowledgments: We thank Ron Desrosiers and the AIDS Research and Reference Reagent Program for the SIV_{mac}239 infectious clone. We thank Michael Wyand and colleagues at the TSI Mason Laboratories for help with the animal studies and for useful discussions. We thank Ginny Nixon and Jan Welch for manuscript preparation and Amy Emmert for artwork. This work was made possible by a gift from William McCarty-Cooper and by small individual gifts to the Division of Human Retrovirology at Dana-Farber Cancer Institute. This work was also supported by a National Cooperative Drug Discovery Grant (AI24845) and a Center for AIDS Research Grant to the Dana-Farber Cancer Institute.

REFERENCES

1. Barre-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic virus from a patient at risk for acquired immunodeficiency syndrome (AIDS). *Science* 1983;220:868-71.
2. Gallo RC, Salahuddin SZ, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 1984;224:500-3.
3. Clavel F, Guetard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. *Science* 1986;233:343-6.
4. Desrosiers RC. The simian immunodeficiency viruses. *Ann Rev Immunol* 1990;8:557-8.
5. Desrosiers RC, Daniel MD, Li Y. HIV-related lentiviruses of nonhuman primates. *AIDS Res Hum Retrovir* 1989;5:465-73.
6. Alter H, Eichberg J, Masur H, et al. Transmission of HTLV-III infection from human plasma to chimpanzees: an animal model for AIDS. *Science* 1984;226:549-52.
7. Fultz PN, McClure HM, Swenson RC, et al. Persistent infection of chimpanzees with human T-lymphotropic virus type III/lymphadenopathy-associated virus: a potential model for acquired immunodeficiency syndrome. *J Virol* 1986;58:116-24.
8. Fultz P, McClure H, Stevenson R. Persistent infection of chimpanzees by HTLV-III/LAV: a potential model for AIDS. *Science* 1986;226:549-52.
9. Gajdusek DC, Amyx HL, Gibbs CJ Jr., et al. Infection of chimpanzees by human T-lymphotropic retroviruses in brain and other tissues from AIDS patients. *Lancet* 1985;1:55-6.
10. Nara PL, Robey WG, Arthur LO, et al. Persistent infection of chimpanzees with human immunodeficiency virus: serological responses and properties of reisolated viruses. *J Virol* 1987;61:3173-80.
11. Daniel MD, Letvin NL, King NW, et al. Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 1985;228:1201-4.
12. Kestler H, Kodama T, Ringler D, et al. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* 1990;248:1109-12.
13. Letvin NL, Daniel MD, Sehgal PK, et al. Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* 1985;230:71-3.
14. Berman P, Gregory T, Riddle L, et al. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. *Nature* 1990;345:622-5.
15. Emini E, Schlieff W, Nunberg J, et al. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. *Nature* 1992;355:728-30.
16. Hu SL, Abrams K, Barber G, et al. Protection of macaques against SIV infection by subunit vaccines of SIV envelope glycoprotein gp160. *Science* 1992;255:456-9.
17. Burns DPW, Desrosiers RC. Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. *J Virol* 1991;65:1843-54.
18. Overbaugh J, Rudensey LM, Papenhausen MD, Benveniste RE, Morton WR. Variation in SIV *env* is confined to V1 and V4 during the progression to simian AIDS. *J Virol* 1991;65:7025-31.
19. Berkower I, Smith G, Giri C, Murphy D. Human immunodeficiency virus 1: predominance of a group-specific neutralizing epitope that persists despite genetic variation. *J Exp Med* 1989;170:1681-95.
20. Dalgleish AG, Beverly PCL, Clapham PR, Crawford DG, Greaves ME, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retroviruses. *Nature* 1984;312:763-7.
21. Haigwood N, Barker C, Higgins K, et al. Evidence for neutralizing antibodies directed against conformational epitopes of HIV-1 gp120. *Vaccines* 1990;90:313-20.
22. Ho D, McKeating J, Li X, et al. Conformational epitope on gp120 important in CD4 binding and human immunodeficiency.

- cy virus type 1 neutralization identified by a human monoclonal antibody. *J Virol* 1991;65:489-93.
23. Kang C-Y, Nara P, Charnat S, et al. Evidence for non-V3-specific neutralizing antibodies that interfere with gp120/CD4 binding in human immunodeficiency virus-infected human. *Proc Natl Acad Sci USA* 1991;88:6171-5.
 24. Klatzmann D, Champagne E, Charnat S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature (London)* 1984;312:767-8.
 25. McDougal JS, Nicholson J, Cross G, Cort S, Kennedy M, Mawle A. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *J Immunol* 1986;137:2937-44.
 26. Posner M, Hideshima T, Cannon T, Mukherjee M, Mayer K, Byrn R. An IgG human monoclonal antibody which reacts with HIV-1 gp120, inhibits virus binding to cells, and neutralizes infection. *J Immunol* 1991;146:4325-32.
 27. Profy A, Salinas P, Eckler L, Dunlop N, Nara P, Putney S. Epitopes recognized by the neutralizing antibodies of an HIV-1-infected individual. *J Immunol* 1990;144:4641-7.
 28. Steimer KS, Scandella CJ, Skiles PV, Haigwood NL. Neutralization of divergent HIV-1 isolates by conformation-dependent human antibodies to gp120. *Science* 1991;254:105-8.
 29. Tilley SA, Honnen WJ, Racho M, Hilgartner M, Pinter A. A human monoclonal antibody against the CD4 binding site of HIV-1 gp120 exhibits potent, broadly neutralizing activity. *Res Virol* 1991;142:247-59.
 30. Thali M, Olshevsky U, Furman C, Gabuzda D, Posner M, Sodroski J. Characterization of a discontinuous HIV-1 gp10 epitope recognized by a broadly neutralizing human monoclonal antibody. *J Virol* 1991;65:6188-93.
 31. Thali M, Furman C, Ho DD, et al. Discontinuous, conserved neutralization epitopes overlapping the CD4 binding region of the HIV-1 gp120 envelope glycoprotein, submitted for publication.
 32. McKeating J, Thali M, Furman C, et al. Amino acid residues of the human immunodeficiency virus type 1 gp120 critical for the binding of rat and human neutralizing antibodies that block the gp120-CD4 interaction. *Virology* (in press).
 33. Javaherian K, Langlois A, Bolognesi D, LaRosa G, Putney S. The principal neutralizing determinant of SIV is highly conformation-dependent and can not be produced by small linear peptides. In: Girard M, Vallette L, eds. *Sixieme colloque des-cent gardes*, Paris: Pasteur Vaccins, 1991:161-4.
 34. Weiss RA, Clapham PR, Weber JN, Dalglish AG, Lasky LA, Berman PW. Variable and conserved neutralization antigens of human immunodeficiency virus. *Nature (Lond)* 1986;324:572-5.
 35. Kestler HW III, Ringler DJ, Mori K, et al. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. *Cell* 1991;65:651-62.
 36. Fisher A, Collalti E, Ratner L, Gallo RC, Wong-Staal F. An infectious molecular clone of HTLV-III. *Nature* 1985;316:262-6.
 37. Rho H, Poiesz B, Ruscetti F, Gallo RC. Characterization of the reverse transcriptase from a new retrovirus (HTLV) produced by a human cutaneous T-cell lymphoma cell line. *Virology* 1981;112:355-60.
 38. Jawetz E, Melnick JL, Adelberg E. Serologic diagnosis and immunologic detection of virus infections. *Review of medical microbiology*, 14th ed., Los Altos, CA: Lange Medical Publications, 1980:371-85.
 39. Miller MD, Lord CI, Stallard V, Mazzara GP, Letvin NL. Gag-specific cytotoxic T lymphocytes in rhesus monkeys infected with the simian immunodeficiency virus of macaques. *J Immunol* 1990;144:122-8.
 40. Letvin N, Lord C, King N, Wyard M, Myrick K, Haseltine W. Risks of handling HIV. *Nature* 1991;349:573.
 41. Guyader M, Emerman M, Sonigo O, Clavel F, Montagnier L, Alizon M. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature (Lond)* 1987;326:662-9.
 42. Viglianti GA, Mullins JI. Functional comparison of transactivation by simian immunodeficiency virus from rhesus macaques and human immunodeficiency virus type 1. *J Virol* 1988;62:4523-32.
 43. Cohen EA, Terwilliger E, Sodroski J, Haseltine W. Identification of a protein encoded by the *vpu* gene of HIV-1. *Nature* 1988;344:532-4.
 44. Klimkait T, Strebel K, Hoggan MD, Martin MA, Orenstein JM. The human immunodeficiency virus type 1-specific protein *vpu* is required for efficient virus maturation and release. *J Virol* 1990;64:621-9.
 45. Strebel K, Klimkait T, Maldarelli F, Martin M. Molecular and biochemical analysis of human immunodeficiency virus type 1 *vpu* protein. *J Virol* 1989;63:3784-91.
 46. Strebel K, Klimkait T, Martin M. A novel gene of HIV-1, *vpu* and its 16-kilodalton product. *Science* 1988;241:1221-3.
 47. Terwilliger EF, Cohen EA, Lu Y, Sodroski JG, Haseltine WA. Functional role of human immunodeficiency virus type 1 *vpu*. *Proc Natl Acad Sci USA* 1989;86:5163-7.
 48. Willey R, Maldarelli F, Martin M, Strebel K. HIV-1 *vpu* protein regulates the formation of intracellular gp160-CD4 complexes. *J Virol* 1992;66:226-34.
 49. Henderson LE, Sowder RC, Copeland TD, Benveniste RE, Oroszlan S. Isolation and characterization of a novel protein (X-ORF product) from SIV and HIV-2. *Science* 1988;241:199-201.
 50. Hu W, Vander Heyden N, Ratner L. Analysis of the function of viral protein X (VPX) of HIV-2. *Virology* 1989;173:624-30.
 51. Kappes JC, Conway JA, Le S-W, Shaw GM, Hahn BH. Human immunodeficiency virus type 2 *vpx* protein augments viral infectivity. *Virology* 1991;184:197-209.
 52. Salter RD, Howell DN, Cresswell P. Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. *Immunogenetics* 1985;21:235-46.
 53. Kanki P, McLane M, King N, et al. Serological identification and characterization of a macaque T-lymphotropic retrovirus closely related to HTLV-III. *Science* 1985;228:1199-1201.
 54. Hattori N, Michaels F, Fargnoli K, Marcon L, Gallo RC, Franchini G. The human immunodeficiency virus type 2 *vpr* gene is essential for productive infection of human macrophages. *Proc Natl Acad Sci USA* 1990;87:8080-4.
 55. Shibata R, Miura T, Hayami H, et al. Mutational analysis of the human immunodeficiency virus type 2 (HIV-2) genome in relation to HIV-1 and simian immunodeficiency virus SIV_{AGM}. *J Virol* 1990;64:742-7.
 56. Yu X-F, Matsuda M, Essex M, Lee T-H. Open reading frame *vpr* of simian immunodeficiency virus encodes a virion-associated protein. *J Virol* 1990;64:5688-93.
 57. Shibata R, Kawamura M, Sakai H, et al. Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J Virol* 1991;65:3514-20.